26. The essentially pure, intact hPTH of claim 23, wherein the genetically engineered microorganism is yeast.

27. An intact, recombinant hPTH which is fully active in an adenylate cyclase assay.

28. The intact, recombinant hPTH of Claim 27 wherein said hPTH is essentially pure.

IN THE TITLE

Please delete the current title "Production of Human Parathyroid Hormone From Microorganisms" and insert in place thereof, --Human Parathyroid Hormone.--

IN THE SPECIFICATION

Please amend the specification as follows:

On Page 1, please delete line 20 beginning with the word "This" through line 23, ending with the word "abandoned."

On Page 1, line 6, after "1993", insert -- now U.S. Patent No. 5,420,242 issued May 30, 1995--.

On Page 1, line 7, after "1993", insert --,now abandoned--.

On Page 8, line 25, please delete "Figure 8. Analysis" an insert --Figures 8a-8c show analysis--.

On Page 9, line 1, please delete "Figure 9. Purification" and insert in place thereof --Figures 9A - 9D show the purification -- .

On Page 9, line 28, please delete "Figure 13. Purity" and insert in place thereof --Figures 13A and B show the purity--.

Remarks

Entry of the foregoing and reexamination and reconsideration of the above captioned application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, and in light of the remarks which follow, are respectfully requested.

As a preliminary matter, the undersigned and Mr. Bruce Sales, Applicants' legal representatives, wish to thank Dr. Spector for the courtesies extended by her during an interview in her office on February 21, 1996. During that interview, the undersigned explained the deficiencies of the isolation and synthetic techniques described in the art identified by the Examiner in the Official Action. The undersigned also presented evidence in the form of photographs of electrophoretic gels showing the difference in purity

between recombinant hPTH produced in accordance with the present invention and synthetically produced materials. The Examiner was also shown a copy of a 1994 article in the refereed journal *Peptides* showing direct *in vitro* and *in vivo* comparative evidence between the recombinant hPTH of the present invention and the best synthetically produced material available today.

The Examiner acknowledged that such evidence was persuasive of differences between the claimed subject matter and the art of record.

Applicants also provided the Examiner with a copy of some draft claims and requested the Examiner's comments regarding same. The Examiner will note that in the draft claims presented during the interview, claim 1 was canceled in favor of claim 3. However, in the amendment proffered hereby, claim 1 is retained while claim 3 is canceled. The reason for this change is that the term "essentially pure" recited in claim 1 finds literal support in the specification at least at page 7, line 25 and is defined in the paragraph preceding that line. The term also finds implicit and inherent support throughout the specification, and in particular, Example 8, pg. 19, lines 7-10, pg. 5, lines 1-6, pg. 3, lines 17-24, pg. 4, lines 15-19, pg. 14, lines 9-14 and pg. 34, line 20 through pg. 35, line 5 to cite but a few passages. Applicants believe that the terms "substantially pure" and "essentially pure" are interchangeable in the context of the present invention. Therefore, Applicants have opted to elect a claim which finds literal support within the specification. This is consistent with comments made by the Examiner during the interview regarding identifying support for claimed terms.

Consistent with the foregoing, claims 23, 24, 25 and 26 have also been changed from "substantially" to "essentially". Literal support for newly added claims 27 and 28, not presented at the interview, can be found in the specification at page 7, lines 32-35. Again, however, implicit support is found throughout the specification, and in particular, at Example 8, pg. 19, lines 7-10, pg. 5, lines 1-6, pg. 3, lines 17-24, pg. 4, lines 15-19, pg. 14, lines 9-14 and pg. 34, line 20 through pg. 35, line 5. Applicants also respectfully submit that claims 23, 24, 25 and 26 are supported

throughout in the specification as a whole, and in particular at pg. 7 lines 32-35. Claims 21, 22 and 24 now depend from claim 1.

Turning to the Official Action, Applicants first acknowledge the Examiner's designation of the Restriction Requirement as being "Final".

The specification was objected to 37 C.F.R. § 1.75(d)(1) and M.P.E.P. § 608.01(l). The Examiner indicated that correction of the specification was required as there was no antecedent basis in the specification for the claim recitations 90% or 95% purity. Applicants respectfully submit that there is literal support for these terms. Nonetheless, as the claims at issue have been canceled hereby, the objection is believed to be moot. Should the Examiner consider it helpful for Applicants to identify where in the specification such literal language can be found, Applicants are willing to do so.

The Examiner also objected to the title of the invention as not being sufficiently descriptive. Accordingly, Applicants have deleted the title and proffered a new title which identifies the subject matter of the claimed invention. Should the Examiner require additional corrections, Applicants would appreciate the Examiner's assistance in drafting a title which is sufficiently descriptive.

The Examiner has asked that the status of all applications to which references are made in the first paragraph of the application be updated and has suggested that the information on page 1, lines 20-23 is duplicative of the first paragraph. The Examiner is correct. The duplicative passages have been deleted. The status of the applications identified in the first paragraph on page 1 has been updated.

The Examiner objected to certain of the Brief Descriptions of the Drawings noting that they should reflect each individual panel of each figure. Appropriate corrections have been made. Should the Examiner require any additional change thereto, Applicants would request the Examiner's assistance in identifying specific language which would be considered acceptable.

Applicants also acknowledge the Examiner's objections to Figs. 6, 7 and 10. Applicants will offer such corrections

as are necessary once there has been an indication of allowable subject matter. Should this present a problem, Applicants respectfully request that the Examiner so inform the undersigned and expedited steps will be taken to address the figures.

Claims 1-5, 11, 13 and 15 stand rejected pursuant to the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 6 of U.S. Patent No. 5,010,010. Applicants will not traverse the rejection and instead will offer, once allowable subject matter is indicated, a properly executed terminal disclaimer. U.S. Patent No. 5,010,010 is commonly assigned with this application. Accordingly, a terminal disclaimer should, at least provisionally, render moot the double patenting rejection. Should the Examiner require the submission of a terminal disclaimer prior to an indication of allowable subject matter, Applicants will attempt to expedite same. The Examiner's indulgence with regard to the filing of such formal papers is appreciated since the inventors and assignees of the application reside outside the United States.

The Official Action contained a number of specific objections and rejections pursuant to 35 U.S.C. § 112, 1st and 2d paragraphs. Applicants believe that the claims, as amended, render moot those objections and rejections. Specifically, Applicants have amended the application so that it no longer claims "a synthetic" peptide and no longer claims both an "essentially pure" and a "substantially pure" peptide. Applicants have also amended the product-by-process claims so that they now explicitly require a purification step. Again, should the Examiner have any specific questions with regard to any of the language used in the claims, she should feel free to contact the undersigned.

The claims, prior to amendment, were rejected pursuant to 35 U.S.C. § 102(b) over *Kumagaye et al.*, *Kimura et al.*, *Fairwell et al.* and *Brewer et al.* The same claims were rejected over the same four references, when considered pursuant to 35 U.S.C. § 103. Applicants respectfully traverse these rejections as applied to the amended claims.

As discussed during the interview, and for the convenience of the Examiner, Applicants will keep their

comments with regard to the prior art to a minimum. Instead, Applicants respectfully request that the Examiner consider the detailed comments of Dr. John Maggio and Dr. Kaare M. Gautvik contained in their concurrently filed declarations. As appropriate, Applicants have referenced the pertinent paragraphs of the declarations to their discussion. Of course, if the Examiner has any questions regarding the declarations or requires further comments of Applicants, she should contact the undersigned.

Brewer et al. relate to the isolation of, primarily, the 34 amino acid N-terminal region of hPTH. *Brewer et al.* do not teach a recombinant product. (Maggio Decl. ¶ 9). Moreover, Fig. 1 of *Brewer et al.* contain three errors in the amino acid of the first 34 amino acids. (Maggio Decl. ¶ 9). This calls into question whether or not *Brewer et al.* actually produced an intact hPTH. (Maggio Decl. ¶ 9). The purification technique used by *Brewer et al.* was gel filtration followed by ion exchange chromatography.

Kimura et al., a later-dated reference cited by the Examiner, clearly demonstrates that *Brewer et al.'s* purification techniques were inadequate. In Fig. 2, *Kimura et al.* show a chromatogram of hPTH from *Brewer et al.* purified by gel filtration and ion exchange chromatography. (Maggio Decl. ¶ 9). Impurities are clearly evident. (Maggio Decl. ¶ 9). To obtain their own purification, *Kimura et al.* employed an additional reverse phase-high pressure liquid chromatography (RP-HPLC) step not employed by *Brewer et al.* The need for the RP-HPLC step further evidences the inadequacies of the *Brewer et al.* protocol. (Maggio Decl. ¶ 9).

Fairwell et al. suffers from many of the same problems as *Brewer et al.* (Maggio Decl. ¶ 10). Like *Brewer et al.*, *Fairwell et al.* employed a separation protocol based on gel filtration followed by ion exchange chromatography. Therefore, Applicants' prior comments with regard to *Kimura et al.* are equally cogent here. (Maggio Decl. ¶ 10). Additionally, *Fairwell et al.* produced a point mutant of hPTH; Asp in position 76 instead of Asn. (Maggio Decl. ¶ 10).

Kimura et al. do not teach essentially pure hPTH. In *Kumagaye et al.*, which is a later paper by the *Kimura et al.*

group, the investigators acknowledged that "[t]oday, many peptides are synthesized by a solid-phase procedure and purified simply by a RP-HPLC system; the present results clearly indicate that the purification of synthetic peptides by RP-HPLC [the procedure used by *Kimura et al.*] is not sufficient to obtain homogeneous products." *Kumagaye et al.* at 330. (Emphasis added) (Maggio Decl. ¶ 11). In fact, *Kimura et al.* acknowledge that they were not able to resolve native hPTH from its Asp^{76} point mutant. *Kimura et al.* at 498.

Finally, *Kumagaye et al.* does not provide sufficient basis for one of ordinary skill in the art to conclude that essentially pure hPTH was produced. (Maggio Decl. ¶ 12). Although *Kumagaye et al.* appears to have resolved hPTH from the Asp^{76} point mutant, purity is not demonstrated. The results only mean that the *Kumagaye et al.* material was free of the Asp^{76} point mutation. (Maggio Decl. ¶ 12). No other inference can be drawn. In fact, if an inference were to be drawn at all, the inference would be that the resulting material was not essentially pure because of the well-known problems associated with solid phase chemistry. (Maggio Decl. ¶¶ 12, 17).

During the interview, the Examiner acknowledged what the art recognized; namely, that there are significant impurity problems with synthetic, solid phase protocols such as those disclosed in *Kumagaye et al.*, *Kimura et al.* and *Fairwell et al.* Premature chain termination, omitted coupling steps, and double coupling steps are widely known and frequently occurring problems. (Maggio Decl. ¶ 15). *See also Fairwell et al.* at page 2691. In addition, synthetic protocols, including these employing the use of BOC chemistry, are known to produce racemization. (Maggio Decl. ¶ 14). For example, *Kimura et al.* describe possible contamination by a $D\text{-}Glu^{22}$ containing peptide. The incidence of these types of impurities increases exponentially with the length of the peptide and a peptide of 84 amino acids in length, such as hPTH is considered long, even by today's standards. (Maggio Decl. ¶¶ 14, 15 and 16). Therefore, those of ordinary skill in the art would expect the presence of impurities based on the type of solid phase chemistry employed in the references cited. (Maggio Decl. ¶ 17).

Moreover, as those of ordinary skill in the art appreciate, unless all of the impurities produced possess a sufficient charge differential, they cannot be separated from intact hPTH using of the techniques described in the art of record. (Maggio Decl. ¶ 16). Consequently, one of ordinary skill in the art would be prone to speculate that the material produced in accordance with, for example, *Kumagaye et al.*, would contain many of these impurities, impurities which would co-elute with hPTH. (Maggio Decl. ¶ 17).

For the reasons just explained, Applicants believe that it would be improper for one to conclude that any of the references applied in the aforementioned Official Action teach or suggest the production of essentially pure hPTH. On that basis alone, Applicants believe that the various prior art rejections are fully met. Nonetheless, during the interview, the Examiner expressed her opinion that despite the references' shortcomings, Applicants would still need to establish that the recombinant hPTH of the present invention was superior, in terms of its claimed attributes, when compared to synthetically produced material.

The evidence necessary to establish the superiority of the present invention can be found in the exhibits attached to Dr. Gautvik's declaration and in the comments of Drs. Gautvik and Maggio. Again, for the convenience of the Examiner, Applicants will not belabor the record reexplaining that which is eloquently set forth by the declarants. Instead, the undersigned will merely summarize some of the more noteworthy points of the declarations. Should the Examiner feel that she would benefit from further explanation, please contact the undersigned.

All of the electrophoretic gels and data presented in the refereed *Peptides* paper, attached to Dr. Gautvik's declaration, were obtained using hPTH material produced in the mid- to late 1980s following the exact protocols disclosed in the above-captioned application. (Gautvik Decl. ¶ 5). The only purification steps used were those described in the application itself. (Gautvik Decl. ¶¶ 5, 10). Applicants respectfully submit that the continuing viability of that batch of material, over the many years since its recombinant

synthesis, speaks volumes with regard to its purity. (Gautvik Decl. ¶ 9).

As shown in Glossy 0 (Gautvik Decl. Ex. B), hPTH produced in accordance with the present invention migrated as a single bright band. (Lane 2, second from the left). Commercially available synthetic material from Sigma, shown in Lane 3 (third lane from left), includes two distinct bands of impurities of higher molecular weight than the hPTH of the present invention. (Gautvik Decl. ¶ 6; Maggio Decl. ¶ 20). The relative intensities and breadth of the bands of impurities are significant because each lane was originally loaded with an identical amount of peptide material. (Maggio Decl. ¶ 20).

Glossy III (Gautvik Decl. Ex. E) shows a comparison between electrophoretic gel recombinant hPTH and what is considered to be the best available synthetic material from the supplier Bachem. The gel distinctly shows a smear of lower molecular weight impurities. (Maggio Decl. ¶ 21; Gautvik Decl. ¶ 9). Additionally, Glossy II (Gautvik Decl. Ex. D) shows that even at a loading of 200 nanograms, very little Bachem material was discernible while strong, broad bands of recombinant material are clearly evident. (Maggio Decl. ¶ 22; Gautvik Decl. ¶ 8).

Applicants draw the Examiner's attention to the paper entitled "Differences in Binding Affinities of Human PTH(1-84) Do Not Alter Biological Potency; a Comparison Between Chemically Synthesized Hormone, Natural Mutant Forms", *Peptides* (1994) Vol. 15, No. 7, pgs. 1261-1265 ("the *Peptides* paper"), a copy of which is attached to Dr. Gautvik's declaration as Exhibit F. This work, undertaken by Dr. Gautvik and his coworkers, was published in 1994. This paper is significant because of the consistency of the results reported with the electrophoretic evidence just discussed. Moreover, as Dr. Maggio points out in his declaration, the paper is greatly pertinent because of its rigorous statistical treatment of the subject matter (provision of 95% confidence intervals and its repetitive testing in triplicate) and because of the variety of testing methods utilized. (Maggio Decl. ¶ 24). Coupled with the variety of methods reported in the specification of the above-captioned application, this article provides a high degree of confidence with regard to the correlation between the gels, the

testing reported in the *Peptides* article and the testing reported in the application.

Fig. 1 of the *Peptides* paper shows the inhibition of radiolabeled [Tyr^{36}] chicken PTHrP(1-36) amide by different hPTHs. As shown in the figure and as explained on page 1264, chemically synthesized hPTH had a calculated binding affinity (K_d) of 18nM while the recombinant hPTH from both yeast and *E. coli* had a significantly lower apparent K_d of 9.5nM. This represents an almost two fold difference between the binding affinity of the recombinant hPTH in accordance with the present invention when compared to the material tested -- the best available synthetic material from Bachem. (Maggio Decl. ¶ 24). The provision of 95% confidence intervals further enhances the high statistical significance of this data. (Maggio Decl. ¶ 24).

Biological activity also supports the purity of Applicant's hPTH. As shown in Fig. 2 and explained on page 1264 of the *Peptides* paper, the recombinant hPTH of the present invention has significantly higher biological activity compared to material produced by solid phase chemical synthesis. Specifically, the recombinant hormone exhibited an almost four fold increase in its ability to stimulate intracellular cAMP accumulation. The EC_{50} values for synthetic hormone were 1.5nM while the recombinant material exhibited an EC_{50} of only 5.7nM. (Maggio Decl. ¶ 25; Gautvik Decl. ¶ 12). Perhaps more importantly, and also as shown in Fig. 2, recombinant hPTH in accordance with the present invention achieved a higher maximal response or efficacy when compared to the best available synthetic material. (Maggio Decl. ¶ 26; Gautvik Decl. ¶ 12). This means that no amount of synthetic material could provide the same efficacy as a maximal dose of recombinant hPTH. (Maggio Decl. ¶ 26; Gautvik Decl. ¶ 12).

The *in vivo* evidence presented in the *Peptides* paper is equally compelling. For the sake of brevity, Applicants merely point to the hypercalcemic assay illustrated in Fig. 3 and discussed on page 1264. In order to achieve an effect almost equal to that of 2.0 micrograms of recombinant hPTH, a 2.7 micrograms dose of chemically synthesized hPTH was required. Thus, the synthetic hPTH had a 30% lower biological activity than the recombinant hPTH. This contrasts markedly

with the maximal response achieved from a considerably lesser dosage of recombinant hPTH. (Maggio Decl. ¶¶ 26, 27).

As the foregoing data compellingly establish, the purity of the recombinant hPTH of the present invention is vastly superior to that of synthetic peptide. This is demonstrated by gels equally loaded with recombinant and synthetic hormone in which synthetically produced hormone had both impurities and significantly lower band intensities; by *in vitro* biological testing which showed that recombinant hPTH exhibited a two-fold increase in affinity, a four-fold increase in biological potency and a higher maximal response or efficacy than could be achieved by the synthetic peptide; and by *in vivo* testing of dosage levels and maximal response studies in which the recombinant hormone was constantly superior to the synthetic hormone.

The recombinant hPTH of the present invention is superior not only in terms of its quantity but also in terms of its qualities. (Maggio Decl. ¶ 27). Because the hPTH of the present invention is produced by recombinant technology, it does not suffer from many of the known disadvantages of synthetic production. Those of ordinary skill in the art would clearly understand this to be the case based on their knowledge of the shortcomings of solid phase synthesis and the fact that the cellular editing mechanisms available in microorganisms such as yeast and *E. coli* would all but eliminate the inherent impurities created during synthetic synthesis. (Maggio Decl. ¶ 17). Therefore, those of ordinary skill in the art would understand that the term "recombinant" describes not only a process by which the resulting hPTH peptide was produced, but also laudatory characteristics which describe the purity and the functional qualities of the resulting material. At the very least, when such a term modifies the term "essentially pure" it indicates the types of impurities that could not be present in the claimed hPTH.

In summary, Applicants believe that claim 1, as amended, has three specific points of novelty, each of which could independently support a conclusion of patentability. First, the claim recites that the hPTH material obtained must be "essentially pure" as that term is defined in the specification. The supporting evidence presented herein

clearly establishes the overwhelming superiority of the purity of the hPTH material produced in the present invention versus the best available material produced by the techniques described in the art of record. Second, the hPTH produced must be "intact." "Intact" hPTH is structurally identical to the naturally occurring peptide; it is full length and identical in amino acid composition. Finally, the material is novel, and indeed, unobvious because it is produced recombinantly. The use of the term "recombinant" in the context of the present invention clearly provides additional information to those of ordinary skill in the art with regard to the nature and qualities of the hPTH claimed.

Claim 23 which relates to a product-by-process is also novel and unobvious for the same reasons. Novelty resides in the process used for the production and purification of hPTH as recited in the claims. Claim 27 as well as dependent claims 21, 22, 24 through 26 and 28 are also all novel and unobvious. It has been shown that the recombinant hPTH of the present invention is "fully active" in adenylate cyclase assay...." (Page 7, lines 33 and 34), (Gautvik Decl. ¶ 13). *See also* Example 8, pg. 19, lines 7-10, pg. 5, lines 1-6, pg. 3, lines 17-24, pg. 4, lines 15-19, pg. 14, lines 9-14 and pg. 34, line 20 through pg. 35, line 5. As Dr. Gautvik explains in his Declaration, the fact that hPTH exhibits full activity in this assay indicates that the hPTH of the present invention possesses a level of biological activity which is substantially equivalent to naturally occurring hPTH. The cAMP testing described in the *Peptides* paper only serves to confirm the statements made in the specification. (Gautvik Decl. ¶ 13).

Applicants believe that the claims, as amended, are both novel and unobvious and that the claims satisfy all of the requirements imposed by 35 U.S.C. § 112. Therefore, Applicants respectfully request the withdrawal of the current rejections and an indication of the allowability of the presently claimed invention.

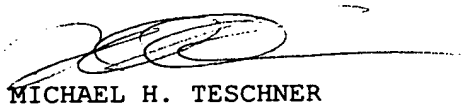
Should Examiner Spector have any questions with regard to the foregoing, she should feel free to contact the undersigned, at her convenience at 908-654-5000. Furthermore, should any fee be due and owing regarding this matter, the

Examiner is hereby authorized to charge Deposit Account No. 12-1095 therefor.

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order and such action is earnestly solicited.

Respectfully submitted,

LERNER, DAVID, LITTENBERG,
KRUMHOLZ & MENTLIK



MICHAEL H. TESCHNER
Reg. No. 32,862

600 South Avenue West
Westfield, NJ 07090
(908) 654-5000 - Telephone
(908) 654-7866 - Facsimile

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